version of the experiments was evidently due to changes in the circadian rhythm of mitosis [9] or other mechanisms [6]. It must be emphasized in particular that the PM level of stressed rats receiving *Schizandra* lignans was the same as in the intact control. In the lingual epithelium of the animals administration of the lignans led to normalization of the number of DNA-synthesizing nuclei, but LI remained increased.

Elevation of the PM level during exposure to stress exceeding the powers of adaptation of the animal, and also in the decompensated version of the GAS, was interpreted by the writers as a structural trace of disadaptation [10]. The ability of *Schizandra* lignans to prevent elevation of the PM level in stress is interpreted as a cellular manifestation of its adaptive properties. The absence of activation of DNA synthesis in the corneal epithelium, and also the weakening of this effect in the tongue can be explained as follows. By alleviating the course of the GAS during chronic exposure to cold, the *Schizandra* preparation weakened the harmful effect of stress, reduced cytolysis, and thereby abolished a powerful stimulus for cell proliferation. However, this hypothesis requires further experimental analysis.

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INDUCTION OF SENSITIVITY OF FIBROBLAST CULTURES TO PITUITARY GROWTH HORMONE BY A THERMOSTABLE SERUM FACTOR

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Many facts have now been published that have led to a critical attitude toward the generally held view that the mechanism of stimulation of growth processes by pituitary growth hormone (GH) is mediated by somatomedins. The direct effect of GH on growth of certain cells in culture [10] and also on growth of cartilage and bone tissue [11] has been described. Specific receptors for GH have been found in chondrocytes [9]. Acceptance of the direct growth-stimulating effect of GH on cells, it must be pointed out, does not exclude participation of somatomedins in the mechanism of its action, but through local (intracellular or intratissue) and not by distant mediators.

The possibility of stimulation of growth processes by GH in cell cultures provides the investigator with a convenient model with which to study the growth-stimulating activity of GH *in vitro*. To study the mitogenic activity of various growth factors cultures of fibroblasts are widely used [4, 8]. It has been shown that preparations of GH belonging to dif-

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TABLE 1. Effect of GH and of Thermostable and Acid-Resistant Fraction of Rat Blood Serum (TSF) on DNA Biosynthesis in Cultures of Human Fibroblasts (M ± m)

Expt. No.	Exptl. conditions	Concn. of TSF, %	Concn. of GH, M	No. of coverslips	Incorp. of ^{3}H-thymidine, cpm
1	Control	—	—	6	122±15
	TSF of intact rats	1	—	6	195±29
		5	—	6	248±37 †
	TSF of hypophysectomized rats	1	—	6	193±20*
	Human GH	—	10^{-6}	6	106±29
		—	10^{-7}	6	152±21
	Human GH + TSF of intact rats	1	10^{-6}	6	135±18
		1	10^{-7}	4	119±11
	Human GH + TSF of hypophysectomized rats	1	10^{-6}	6	406±113*
		1	10^{-7}	4	222±20 †
2	Control	—	—	6	191±54
	TSF of hypophysectomized rats	1	—	6	133±32
		2	—	6	211±83
		5	—	6	248±39
	Human GH	—	10^{-6}	6	229±82
		—	10^{-9}	6	256±127
	Human GH + TSF of hypophysectomized rats	1	10^{-6}	6	458±175 †
		1	10^{-9}	6	230±57
3	Control	—	—	6	113±12
	TSF of intact rats	1	—	6	142±8
		2	—	6	388±110*
	TSF of hypophysectomized rats	1	—	6	164±50
		2	—	6	271±38 †
	Human GH	—	10^{-6}	6	149±16
	Human GH + TSF of intact rats	2	10^{-6}	6	268±25 ‡
	Human GH + TSF of hypophysectomized rats	1	10^{-6}	6	266±60*
		2	10^{-6}	6	365±45 ‡
4	Control	—	—	8	178±50
	TSF of intact rats	1	—	4	213±10
	TSF of hypophysectomized rats	1	—	4	182±51
	Human GH	—	$0{,}5 \cdot 10^{-6}$	6	129±14
	Human GH + TSF of intact rats	1	$0{,}5 \cdot 10^{-6}$	6	295±40

TABLE 1 (continued)

Expt. No.	Exptl. conditions	Concn. of TSF, %	Concn. of GH, M	No. of cover-slips	Incorp. of ^{3}H-thymidine, cpm
	Human GH+TSF of hypophysectomized rats	1	$0{,}5 \cdot 10^{-6}$	6	349±48*
	Bovine GH	—	10^{-6}	6	212±33
		—	10^{-7}	5	156±24
		—	10^{-8}	6	288±71
	Bovine GH + TSF of intact rats	1	10^{-6}	6	375±58*
		1	10^{-7}	6	304±50
		1	10^{-8}	6	329±26*
	Bovine GH + TSF of hypophysectomized rats	1	10^{-6}	4	399±64*
		1	10^{-7}	6	279±30*
		1	10^{-8}	7	390±41†

Legend. *P < 0.05, †P < 0.01, ‡P < 0.001 compared with control.

ferent species, if added to a culture of embryonic or postnatal human fibroblasts, can stimulate DNA biosynthesis [1, 6, 13]. It has also been shown that GH, by its direct action on cultures of fibroblasts, can stimulate somatomedin production by the cells [7]. However, attempts to obtain a growth-stimulating effect of GH in fibroblast cultures are by no means invariably successful [5, 8]. It is not quite clear why this is so. The presence or absence of an effect of GH in a cell culture is evidently determined by the origin of the cells, the conditions of their preliminary culture and their incubation with the hormone, and the properties of the hormone preparation itself.

This paper gives data to show that highly purified GH preparations, themselves unable to stimulate DNA biosynthesis in cultures of adult human skin fibroblasts, acquire this ability if the cells are treated simultaneously with a factor present in a thermostable and acid-resistant fraction of rat blood serum. Activity of this factor in rat blood serum has been shown to depend on the pituitary, and to increase after hypophysectomy.

EXPERIMENTAL METHOD

Highly purified preparations of human and bovine GH were used. Human GH was isolated by the method in [15], using gel filtration through Sephadex G-100 as the final stage of purification*. Bovine GH was isolated by Li's method in a modification in which the final stage of purification was ion-exhcnage chromatography on a column with Amberlite IRC-5 [3]. Both hormone preparations, as shown by N-terminal amino acid analysis by the reaction with dansyl chloride, were homogeneous and each gave two bands characteristic of the corresponding highly purified hormone preparations on analytical polyacrylamide gel electrophoresis. On biological testing (tibia test) on hypophysectomized rats, both preparations exhibited high growth activity.

Blood serum obtained from intact or hypophysectomized rats of the same age were treated by the method used for determination of somatomedin activity [5, 8]. The serum was diluted four times with 0.9% NaCl solution and pH was adjusted to 5.5 by the addition of 0.1 N HCl, and the sample was kept at 100°C for 15 min in a boiling water bath. The precipitate thus formed was removed by centrifugation and the supernatant was neutralized with 0.1 N NaOH and diluted to the required concentration with Eagle's medium. Hypophysectomy was performed on rats weighing 55-60 g, and blood was taken 14 days later from animals whose gain in body weight was significantly slower than that of intact rats. The blood serum was kept at -15°C.

Fibroblasts were obtained from the cell bank of the Institute of Medical Genetics, Academy of Medical Sciences of the USSR. They were fibroblasts of diploid strain IMG-853 (15th-20th passage), isolated from adult human skin biopsy material [2]. The strain was in the second phase of growth. For cell culture, Eagle's medium with 5% bovine serum and 10%

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human umbilical serum was used. The cells were cultured at 37°C under saturating humidity conditions in an atmosphere of 95% air + 5% Co_2. The cell suspension was seeded in volumes of 2 ml on plastic Petri dishes 40 mm in diameter, with a density of 10^4 to 2×10^4 cells/cm^2. Equal-sized coverslips (12 × 24 mm) were placed on the bottom of the Petri dishes. The nutrient medium in the Petri dishes was changed for medium without serum 16 h after subculture of the cells in the Petri dishes. After 64 h, the medium in the dishes was replaced by fresh medium without serum, containing or not containing (control) the test preparations. To determine the intensity of DNA biosynthesis [^{3}H]thymidine was added to the culture (1 μ Ci/ml, 4.4 Ci/mmole, Prague) after 20 h of subculture. The label was washed out with Hanks' solution 8 h after its addition. The coverslips were fixed with a cold mixture of ethanol and acetic acid (3:1) for 30 min at 4°C. The acid-soluble components were washed twice with cold 5% TCA. The air-dried coverslips with the acid-insoluble cell components were placed in flasks containing toluene scintillator and their radioactivity was counted by means of a β-counter (Intertechnique, France). Incorporation of [^{3}H]thymidine into cell material insoluble in TCA, expressed in cpm per coverslip, was used as a measure of the intensity of DNA biosynthesis.

EXPERIMENTAL RESULTS

As Table 1 shows, the intensity of DNA biosynthesis by fibroblasts in culture, measured as incorporation of [^{3}H]thymidine, was not significantly changed after addition of human or bovine GH alone, in a concentration of 10^{-9}-10^{-6} M, to the nutrient medium without serum. The absence of effect of GH in cultures of human fibroblasts contradicts certain data obtained previously [1, 6, 13] and could be due to two circumstances at least. The first is the properties of the hormone preparations used in the work. It is well established that highly purified GH preparations, besides a quantitatively predominant protein built from 190-191 amino-acid residues, may also contain so-called split isoforms [5, 12, 14]. A modification of this type not only does not reduce the biological activity of GH, but may intensify it or lead to the appearance of new biological activity, and it may therefore play an important physiological role. It has been shown that activation of GH by specific limited hydrolysis of its polypeptide chain can take place even during keeping of the preparation as a result of the action of endogenous proteinases present in it in trace amounts, and possessing high affinity for the hormone [14]. The property of stimulating growth processes on direct contact between GH and cells probably arises only after proteolytic modification of its molecule, taking place in the intact organism during secretion of the hormone from the pituitary or transport to target cells. Thus evidently only GH preparations containing activated isoforms can possess growth-stimulating activity in cell cultures. The second circumstance, preventing manifestation of the growth-stimulating activity of GH in fibroblast cultures, may be low sensitivity of the actual cells used (of their receptors) to the growth-stimulating action of the hormone because of their derivation from an adult donor. Another reason for the absence of an effect of bovine GH in a culture of human fibroblasts could be species specificity of the cell receptors.

According to data in the literature [5, 8], the fraction obtained by heating rat blood serum to 100°C in an acid medium for 15 min contains somatomedins. It is therefore logical to conclude that the addition of this fraction to the culture medium in an adequate amount (in a final concentration of 2-5%) led to stimulation of DNA biosynthesis by the cells, and to a particularly marked degree when blood serum from intact rats was used (Table 1, Expt. 3).

However, the presence of the thermostable and acid-resistant blood serum fraction in the culture medium in a concentration of 1% in most cases was insufficient to cause significant stimulation of DNA biosynthesis. Meanwhile the serum fraction present in the culture medium in a concentration of 1% was able to induce sensitivity of the fibroblasts to both GH preparations.

Human GH, while not exhibiting activity itself, if added to the medium simultaneously with serum fraction from hypophysectomized rats, stimulated DNA biosynthesis by fibroblasts significantly. The increase in [^{3}H]thymidine incorporation under the influence of the hormone together with the serum fraction amounted to 233% (Table 1, Expt. 1; difference compared with action of serum fraction without hormone statistically significant — $P < 0.05$). It is important to note that serum fraction of intact rats of the same age in a concentration of 1% was unable to induce sensitivity of the fibroblasts to human GH.

Bovine GH in the presence of serum fraction in a concentration of 1% also acquired the ability to stimulate DNA biosynthesis by fibroblasts. The stimulating effect was observed over a wide range of doses of the hormone: from 10^{-6} to 10^{-8} M. In this case, however, no clear difference between the action of serum fraction from hypophysectomized and intact rats was observed.

The results on the whole, while confirming the possibility of direct action of GH on cell growth, are evidence that the sensitivity of cells to the growth-stimulating action of the hormone is a controlled process and that the pituitary plays an important role in this control. To judge from its thermostability and acid resistance, the factor found in blood serum, produced under the inhibitory control of the pituitary, can hardly be an enzyme protein activating GH through limited specific proteolysis. It is more likely to be some sort of comparatively low-molecular-weight regulator. During the action of serum factor on cells, even weak affinity of the inactivated form of human GH or bovine GH for receptors of adult human fibroblasts is sufficient to trigger the corresponding biochemical mechanisms in the cells responsible for stimulation of DNA biosynthesis. It is quite possible that activity of the serum factor inducing sensitivity of cells to GH is regulated by the blood GH level; if the level of the hormone is high activity is depressed, if it is low (for example, in the absence of the pituitary) it rises.

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